Operational sex ratio and mating behaviour of the myobatrachid frog
Neobatrachus kunapalari

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Abstract

Matings involving two or more males and a single female (polyandry) are now widely reported in frogs. Polyandry may occur when the sex ratio at breeding sites (the operational sex ratio, OSR) is heavily biased towards males. We report here variation in OSR and the occurrence of polyandrous groupings in the frog, *Neobatrachus kunapalari*. When the OSR had a low male bias conventional amplexed pairs formed with one male and one female. When the OSR was more strongly male biased, groups containing one female and up to seven males formed. At a site with an OSR of 21 females:35 males, mated males were significantly smaller than unmated males but there was no correlation between male and female size in amplexed pairs. We argue that skew in the OSR may favour the facultative appearance of behaviours, *e.g.*, multiple male matings, that raise the risk of sperm competition occurring.

Keywords: operational sex ratio, frogs, amplexus, polyandry, mating behaviour

Introduction

Reports of multiple male frogs in amplexus with a single female occur sporadically in many frog families (Byrne & Roberts 2004). In some cases, these sorts of associations lead to polyandrous fertilisation of eggs from a single clutch (*e.g.*, D'orgeix and Turner 1995; Roberts *et al.* 1999) and polyandry is reasonably suspected in others (*e.g.*, Jennions and Passmore 1993). Many multiple-male, single-female associations in frogs may actually involve a real risk of sperm competition. Byrne *et al.* (2002) reported that the risk of sperm competition was associated with an increase in testis mass in Australian myobatrachid frogs. Their analysis of "risk of sperm competition" was based on a survey of the literature where they extracted data on the likelihood of males coming into close, physical proximity or contact with each other (and females), *e.g.*, as in multiple male amplexus. They found that several *Neobatrachus* species had relatively high testes mass (*N. kunapalari*, testes approximately 0.26% of body mass, range in genus, 0.05 to 1.23% across 10 species, Byrne *et al.* 2002). There are reports of both very dense breeding aggregations (*N. pictus*, Roberts 1978) and multiple-male, single-female aggregations in *Neobatrachus* species (*e.g.*, cover photo of Main 1965 for *N. pelobatoides*) indicating a high risk of sperm competition. In *Crinia georgiana*, density, bias in the operational sex ratio (OSR, ratio of fertilisable females to available males, Emlen and Oring 1977) and the frequency of polyandrous matings are all positively correlated (Byrne & Roberts 2004) suggesting a possible general correlate of polyandry: strong male bias in the OSR leads to males using alternative mating tactics, *e.g.*, group matings, to obtain fertilisations.

Neobatrachus kunapalari is an explosive breeder that breeds after heavy summer and winter rains in the semi-arid region of south-western Australia (Mahony & Roberts 1986; Roberts & Majors 1989). We report here observations of multiple male, single female aggregations in the myobatrachid frog *Neobatrachus kunapalari*, and relate this to variation in the operational sex ratio (OSR) in breeding ponds. We speculate about how variation in OSR might affect mating behaviour in *N. kunapalari*.

Methods

We observed breeding behaviour of *N. kunapalari* at two sites: 13 km north of Kellerberrin on the Trayning Road (16–19 April, 2002) and in a rubbish dump at Hedges, 13 km south south-west of Narembeen (21 February, 1986), both in the semi-arid, grain-growing region of Western Australia. At Kellerberrin, the breeding site was a human-made, clay-lined drainage ditch on gently sloping pastureland, approximately 5 x 1 m and 10 cm deep. Pond water was very turbid and opaque due to suspended clay. Observations were made between 19:00 hrs and 21:00 hrs from 16th April to 19th April, 2002. The Narembeen pond was formed after a heavy, late afternoon-evening thunderstorm. It was a roughly rectangular clay pond approximately 30 m x 5 m x 25–30 cm deep with turbid water. Headtorches with white light were used for making all observations. Amplexed pairs were placed in small plastic containers to collect eggs as they were deposited. Snout-vent length of all frogs collected at Narembeen were measured. At both sites all frogs collected were considered mature. For males this was based on the presence of nuptial pads and/or calling activity. Frogs considered female were carrying mature eggs, lacked nuptial pads, were depositing eggs or they were in amplexus and from their size they appeared gravid. We have never observed small, immature frogs of any *Neobatrachus* species in ponds where breeding was taking place despite a

considerable amount of field work on all species in this genus (Roberts 1997a,b).

Data are presented as means ± standard errors with an α of 0.05 used throughout.

Results

Field Observations

Kellerberrin: on the 16th April 2002, there was a heavy rainfall event that produced 13.5 mm of rainfall in 24 hours. Subsequently, we observed a large breeding chorus where males were calling both in the water and from the damp ground surrounding the pond. From 19:00 to 19:30 h, we observed four single-male matings. From 20:00 – 21:00 hrs, we observed three separate, multiple-male amplexus events comprising five, six and seven males respectively, trying to amplex a single female. In each multiple-male amplexus, one male was in inguinal amplexus, one male around each of the front legs, and the remaining males were vying for position underneath and alongside the other males (Figure 1). On the following night (17th April), after a further 7 mm of rain, during two hours of observation, we saw two single male matings. On the third night (18th April), we observed three single male matings. By the fourth night (19th April) all breeding activity (calling and mating) had ceased and the pond contained numerous egg masses. There was a strong bias to males on 16th April but this was drastically reduced on the following two nights (Table 1).

Narembeen: this pond was observed from 00:30 hrs to 03:30 hrs. At 00:30 hrs there was one amplexed pair. Other pairs formed over the next three hours. Seventeen pairs, four unamplexed females and eighteen unamplexed males were collected between 03:00 hrs and 03:30 hrs (close to dawn: a total of 21 females, 35 males). Sixteen conventional single male, single female pairs formed. A seventeenth pair was approached and touched by a calling male but the calling male did not make any obvious attempt to amplex or displace the amplexed male. The caller left and resumed calling.

At Narembeen, mated males had significantly smaller snout-vent lengths than unmated males (t_{33} = 2.203, p = 0.035; mated males 59.23 mm ± 1.01; unmated males, 62.22 mm ± 0.91). The correlation between snout-vent lengths of mated males and females was not significant (r = 0.150, p > 0.05). The number of eggs deposited per pair averaged 918 ± 102 with a range from 28 to 1616. We do

Figure 1. Amplexed group: one female and four males, Kellerberrin. The head and limbs of the female are obscured but her abdomen is visible between the two males on the left.

not know whether females were depositing eggs before pairs were collected so the average may be an underestimate of actual fecundity if there were some incomplete clutches (*e.g.*, the low of 28).

Operational Sex Ratios

Operational sex ratios and frequencies of multiple male matings are given in Table 1. For the first night at Kellerberrin we calculated separate OSR values for observations from 19.00 – 19.30, and those made between 20:00 hrs – 21:00 hrs, because females were not marked and we could not determine whether the same females were amplexed at both time periods. To calculate the OSR we assumed all frogs present in the pond at the time of observation were available to mate as all females appeared gravid and most males were calling.

Discussion

Our observations document polyandrous mating aggregations that might lead to multiple paternity of single egg clutches in *N. kunapalari*. Our data are consistent with an increased frequency of multiple-male, single females aggregations when the OSR is more heavily male biased if we assume the early data from 16th April reflect initial pairing that is modified over time (Table 1). Our observations are comparable to the multiple-male amplexes in *N. pelabatoides*, illustrated on the cover of Main (1965). We did not follow amplexed pairs through the process of egg deposition and the extra

Table 1

Sex ratio and frequency of single- and multiple-male matings in breeding choruses of *N. kunapalari*.

| | Kellerberrin | | | | Narembeen |
| | 16/4/2002 | | 17/4/2002 | 18/4/2002 | 21/2/1986 |
	19.00–19.30	*20.00–21.00*			
Males	32	32	28	26	35
Females	4	4	15	14	21
OSR	0.125	0.125	0.54	0.54	0.6
Number single matings	4	0	2	3	17
Matings with > 1 male	0	3	0	0	0
% Matings with > 1 male	0	100	0	0	0

males may leave the mating assemblages before eggs are released or never release sperm. Demonstrations that additional males release sperm would require direct collection of sperm, *e.g.*, using condoms (Hettyey & Török 2004), or genetic determination of extra-pair paternity.

Apart from attempting to obtain fertilisations, what else could attendant males be doing? Males may be trying to displace other males and gain exclusive fertilisation but the pairing success of smaller males at Narembeen suggests this is unlikely as we would expect larger males to win fights leading to a large male advantage. In *Bufo bufo*, fighting and displacement of amplexed males over several days shifts the population from initially randomly paired by size to positive, size assortative mating, particularly because large males displace smaller males amplexed with larger females (*cf.* Davies & Halliday 1977). The lack of a significant correlation between the size of mated males and females in *N. kunapalari* also argues against displacement leading to more effective fertilisation in pairs with better matched body sizes (*cf.* Davies & Halliday 1977), as some pairs were likely to already be depositing eggs.

Assuming males release sperm sometime during egg deposition, the frequency of multiple male matings in *N. kunapalari*, 21.5% over two sites, is much lower than in *C. georgiana* where 43.5 % of matings involve two or more males (Roberts *et al.* 1999). Our data suggest that attempts at multiple male amplexus may be a facultative response to male bias in the operational sex ratios as multiple male single females aggregations only occurred when the OSR was strongly male biased (Table 1). This also occurs in *C. georgiana* where the frequency of multiple-male matings and the number of males per mating both rise with increasing male bias in the OSR (Byrne & Roberts 2004). Sullivan *et al.* (1995) describe variations in mating behaviour with increasing density in several frog species. At higher densities males are more mobile and less likely to call to attract mates but they did not report multiple-male, single female amplexes but in these examples it was not clear how density and OSR co-vary. In *C. georgiana*, male bias in OSR and density are positively correlated (Byrne & Roberts 2004).

The comparative analyses of testes mass reported by Byrne *et al.* (2002) suggested the real possibility of sperm competition increasing testes mass in many species. However, the mechanism of sperm competition was not clear: it may be direct competition as in *C. georgiana* (Roberts *et al.* 1999) or by more diffuse competition as suggested for *Rana temporaria* (Laurila and Seppa 1998) and *Bufo americanus* (Kaminsky 1997). In *N. kunapalari*, males amplexed in unconventional positions may release sperm that are sufficiently mobile to achieve at least some fertilisations.

Our data for *N. kunapalari* are preliminary but are consistent with studies on other frogs where increasing chorus density and increasing male bias in the OSR are correlated with shifts in male mating tactics (Byrne & Roberts 2004). Our data also are quantified, natural history observations (rather than anecdotes), coupled with specific data on sperm release or multiple paternity, needed to determine whether sperm competition is relatively common in anurans (as suggested by several recent authors, *e.g.*, Halliday & Verrel 1984; Halliday &

Tejedo 1995, Roberts *et al.* 1999, Byrne *et al.* 2002). Ideally, future studies will experimentally manipulate OSR and follow amplexed pairs until egg deposition is complete to test our interpretations.

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